DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTE TO FILE (BNF0017)

May 22, 1996

Subject: European Corn Borer (ECB)-Protected Corn

Keywords:

Corn, *Zea mays*, *Bacillus thuringiensis* var. *kurstaki* (Btk), *CrylA(b)*, lepidopteran toxicity, Btk protein, *Ostrinia nubilalis*, European Corn Borer (ECB), *Streptomyces viridochromogenes*, glufosinate ammonium tolerant, herbicide tolerant, *pat*, phosphinothricin acetyltransferase (PAT), ampicillin resistance, *AMPr*.

Background

In submissions dated October 25, 1995, February 28, 1996, and May 14, 1996, Northrup King Company and their representatives provided summary information to support their safety and nutritional assessment of their new corn line containing transformation event Bt 11.

Intended Effect and Food/Feed Use

The intended technical effect of this genetic modification of corn plants (*Zea mays*) is to confer resistance to lepidopteran insects, specifically the European Corn Borer (ECB) (*Ostrinia nubilalis*).

According to Northrup King, their corn line, containing transformation event Bt 11, has been modified to express a synthetic version of the truncated *CrylA(b)* gene, which was derived from the *CrylA(b)* gene isolated from *Bacillus thuringiensis* var. *kurstaki* (Btk). The *CrylA(b)* encodes the Btk protein, which is toxic to certain lepidopteran insects upon ingestion.

According to Northrup King, their corn line, containing transformation event Bt 11, also expresses a synthetic version of the *pat* gene, which is similar to the *pat* gene isolated from *Streptomyces viridochromogenes*. The *pat* gene encodes the phosphinothricin acetyltransferase (PAT) protein, which reportedly confers tolerance to the herbicide glufosinate ammonium.

For Northrup King's purpose, PAT expression (herbicide tolerance) functions as a selectable marker for development of the Bt 11 hybrid (insect resistant) corn line.

Molecular Alterations and Characterization

A polyethylene glycol-mediated protoplast transformation method was used by Northrup King to generate the transformation event Bt 11. The intact circular transformation vector, pZO1502, was constructed by inserting synthetic, modified *CrylA(b)* and *pat* genes (with the required regulatory DNA sequences), into appropriate sites of intermediate vectors derived from the base plasmid pUC18. Insertion of a portion of the plasmid, pZO1502, containing truncated *CrylA(b)* and *pat* gene expression cassettes (both with 35S promoters, introns, and NOS terminators), into corn line BG, constituted transformation event Bt 11. One copy of the inserted gene sequences was mapped to a site on the long arm of chromosome 8. The phenotypic characteristics (resistance to ECB and tolerance to glufosinate ammonium) of the S1 and S2 populations indicated that the inserted genes were inherited as a single dominant locus.

The ampicillin resistance gene (*AMPr*), used for molecular manipulations in *Escherichia coli*, was selectively deleted from pZO1502 before its use in the corn transformation. Southern blot analysis of Bt 11 transgenic corn indicated that no *AMPr* gene sequences were present. Polymerase chain reaction (PCR) testing, with specific *AMPr* gene primers, confirmed a complete lack of *AMPr* gene sequences.

Northrup King reported that the truncated *CrylA(b)* and *pat* genes were engineered to optimize their expression in plants. Despite differences in the DNA sequences, the synthetic genes, introduced by transformation event Bt 11, code for proteins identical to the Btk and PAT proteins. Northrup King stated that they observed equivalent insecticidal activity with both the truncated Btk protein expressed in Bt11 corn and the full-length microbial Btk protein.

Regulatory Considerations

According to Northrup King, the inserted DNA is capable of expressing two proteins: 1) Btk; and 2) PAT. The safe use of Bt-toxins as pesticides and the use of selectable markers as pesticidal inert ingredients in the development of pesticide resistant plant varieties are regulated by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Therefore, we have not addressed the safe use of Bt-toxin as a pesticide or the safe use of PAT as a pesticidal inert ingredient.

Nutritional Assessment

GRAIN

Based on the nature of the genetic modification, it was expected that Bt 11 corn would not materially differ in composition from other non-transgenic corn varieties. To confirm this

expectation. Northrup King analyzed the nutrient composition of grain obtained from Bt 11 corn and comparable control lines by standard methods for protein, oil, starch, and crude fiber. Insertion of the *CrylA(b)* and *pat* genes did not significantly alter amounts of these analytes.

SILAGE

Northrup King additionally examined the composition of the whole corn plant for both Bt 11 and control corn lines. Analytes were dry matter, crude protein, available crude protein, acid detergent fiber, neutral detergent fiber, total digestible nutrients, calcium, phosphorous, potassium, and magnesium. Although some statistically significant differences were determined when comparing transgenic to control lines (*e.g.*, acid dietary fiber, nutrient dietary fiber, total digestible nutrients, calcium, potassium), values were not inconsistent with amounts normally present in hybrid field corn.

In addition, Northrup King reports that the insertion of the Bt11 modification had no effect on agronomic performance characteristics in selected modified lines. Traits included yield, root lodging, ear height, pollen shed, disease susceptibility, and plant phenotype.

Conclusions

Northrup King has concluded that corn lines containing transformation event Bt 11 are not materially different in composition, nutrition, and safety from corn currently grown, marketed, and consumed for animal feed or human food. At this time, based on Northrup King's description of its data and analyses, the Agency considers Northrup King's consultation on corn grain (kernels), fodder, and silage, derived from corn lines containing transformation event Bt11, to be complete.

V. Kelly Bunning

V.Kelly Bunning, Ph.D.